

May 1, 1963

Dr. Robert Sinsheimer  
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Dear Bob,

I tried to call you this morning but learned you were sunning yourself on the shores of La Jolla at the Histone Conference. Since I'm leaving tomorrow for the East for about a week I thought it best to write.

Mike has finished his work on the  $\phi$ X hybrid and has begun to organize it for publication. He was planning to talk about these studies at the Cold Spring Harbor Symposium and they of course want a written manuscript submitted at the time of the meeting. Since we talked about and agreed to publish ours and your work together, I was wondering how we would manage this. Do you plan to present any of your data at CSH? Since the CSH paper will not be the complete paper we can still plan to submit our more detailed manuscript along with yours to, for example, JMB. Do you have any idea as to when you plan to write your  $\phi$ X hybrid work up?

With regard to our two sets of data there seems to be two bits of data which were at variance. One of these was the  $T_m$  of the hybrid; Mike tells me that your value was higher than his and in fact higher than our value for  $\phi$ X double-stranded DNA. Since we're not sure these were done under identical conditions, I wondered if we could tell you our conditions and we could get your for comparison. Ours is 0.05 M sodium citrate, pH 7.5, although we have done it over a range of salt concentrations (see enclosed figure). An equally attractive alternative is to send you some  $\phi$ X double-stranded DNA which you might compare at equivalent ionic strengths. Our data show that the  $T_m$  of hybrid is always slightly slower than  $\phi$ X double-stranded. We would be interested in knowing whether the same holds true for  $\phi$ X hybrids you make.

The other point of difference is the results of the experiment to determine if the RNA strand of the hybrid is chased by further replication. Mike described our experiment which was done in two ways. Starting with  $\phi$ X hy (U) replicated with  $\overline{\text{BUTP}}$  and  $\phi$ X hy ( $\overline{\text{BU}}$ ) replicated with UTP.

In each case the hybrid shifts to heavy or light density, respectively. However, when Mike used  $\phi$ Xhy (Udg CMP<sup>32</sup>) and replicated with BUTP and isolated the hybrid in a preparative density gradient, the heavy hybrid (very close to the right density found in the analytical run) contained 25-30% of the original P<sup>32</sup>. Since more of the P<sup>32</sup> was in the light region (the original density) this is not due to failure to copy some of the hybrid molecules. Rather it suggests that either some regions of the duplex were copied conservatively or were not copied at all. I think that our gradient runs might not readily distinguish two hybrids which differ by 25-30% in their content of BU.

The last point I wanted to raise was to beg some  $\phi$ X DNA. I told you I hoped to prepare  $\phi$ X during my stay in Honolulu and I did make several attempts with John Hall's group. On three occasions the culture lysed before it was infected and in only one case did we get a legitimate lysate. I harvested this using the polymer-phase separation that Hall uses but this is only enough for me to use as an inoculum for a large biogen run. This leaves me without any DNA to start our sequence studies. I was hoping that if you could spare some we could get started immediately on the sequence work and at the same time be trying again to grow our own virus for future experiments. Last time you were generous enough to send about 0.5-0.7 ml of  $\phi$ X DNA ( $\sim 36$  OD<sub>260</sub>/ml) and this lasted for some time. I'm hard put to tell you exactly what our minimum requirement is to get started - a rough estimate would be about 10 OD units but if you can spare any more than this I'd deeply appreciate it. I hope this will be the last time I'll have to bum  $\phi$ X DNA from you - in fact after all my intentions to return from Honolulu with a batch of  $\phi$ X DNA I'm somewhat embarrassed to ask you for some now. I'll be coming back from the East by May 10 and I hope we can get started soon after. I'd also appreciate hearing from you about the hybrid papers. If you want some of the  $\phi$ X double-stranded DNA, I'll send it out as soon as we hear from you.

I was glad I did get a chance to see you before I left, although I regret it was so rushed and that I didn't get to hear your seminar.

Best regards from all,

Sincerely,

Paul Berg

PB:cm